

WHAT IS CLAIMED IS:

1. A high-affinity mouse monoclonal antibody to human tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), wherein said monoclonal antibody (a) competitively inhibits the binding of antibody A2 to TNF and (b) binds to a neutralizing epitope of human TNF $\alpha$ .

2. A mouse monoclonal antibody according to claim 1 in detectably labeled form.

3. A chimeric immunoglobulin chain comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region having specificity to human TNF $\alpha$ .

4. A chimeric immunoglobulin chain according to claim 3, wherein said chain is a heavy chain or a light chain.

5. A chimeric immunoglobulin chain according to claim 3, wherein said constant region is of human origin.

6. A chimeric antibody molecule comprising two light chains and two heavy chains, each of said chains comprising at least part of a constant region and at least part of a variable region, said variable region having specificity to human TNF $\alpha$ , said antibody binding with high affinity to a neutralizing epitope of human TNF $\alpha$  *in vivo*.

7. A chimeric antibody according to claim 6 which does not bind to TNF $\beta$ .

8. A chimeric antibody according to claim 6, wherein said variable or constant region is of murine origin.

9. A chimeric antibody according to claim 8, wherein said variable region is derived from a high affinity murine monoclonal antibody which binds to a neutralizing epitope of human TNF $\alpha$ .

10. A chimeric antibody according to claim 9, wherein said murine monoclonal antibody competitively inhibits the binding of A2 or cA2 to TNF $\alpha$ .

5 11. A chimeric antibody according to claim 9, wherein said murine monoclonal antibody is A2.

12. A chimeric antibody according to claim 6 wherein said affinity, measured as an association constant (K<sub>a</sub>), is at least  $1 \times 10^8$  liter/mole.

10 13. A chimeric antibody according to claim 6 wherein said affinity, measured as an association constant (K<sub>a</sub>), is at least  $1 \times 10^9$  liter/mole.

14. A chimeric antibody according to claim 6 which neutralizes human TNF $\alpha$  with an ID<sub>50</sub> of at least about 1  $\mu$ g/ml.

15 15. A chimeric antibody according to claim 6 which neutralizes human TNF $\alpha$  with an ID<sub>50</sub> of at least about 100 ng/ml.

16. A chimeric antibody according to claim 6 which neutralizes human TNF $\alpha$  with an ID<sub>50</sub> of at least about 15 ng/ml.

17. A chimeric antibody according to claim 6 in detectably labeled form.

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18. A monoclonal antibody according to claim 1 in detectably labeled form which is produced by a hybridoma or recombinantly.

19. A monoclonal antibody according to claim 1, wherein said antibody has an antigen binding region which binds residues 87-108, or both 59-80 and 87-108, of hTNF $\alpha$  of SEQ ID NO:1.

20. An antibody according to claim 1, wherein said antibody, fragment or region does not bind to one or more epitopes included in amino acids 11-13, 37-42, 49-57 or 155-157 of hTNF $\alpha$  of SEQ ID NO:1.

21. An anti-TNF antibody, or a fragment or region thereof, having an anti-TNF binding region, or fragment thereof, corresponding to a

(a) murine monoclonal antibody of monoclonal antibody A2;

or

(b) chimeric mouse-human monoclonal antibody, fragment or region of monoclonal antibody cA2.

22. A TNF peptide comprising at least 5 amino acids selected from the group consisting of amino acids residues 87-108 or both residues 59-80 and 87-108 of hTNF $\alpha$  of SEQ ID NO:1, wherein said peptide comprises an epitope of an anti-TNF antibody, fragment or region having anti-TNF biological activity by binding to a TNF sequence other than a receptor binding locus, such that TNF binding to a TNF receptor is substantially inhibited.

23. A TNF peptide according to Claim 22, consisting essentially of 3 to 22 amino acids of at least one of the sequences Tyr-Ser-Gln-Val-Leu-Phe-Lys-Gly-Gln-Gly-Cys-Pro-Ser-Thr-His-Val-Leu-Leu-Thr-His-Thr-Ile, as amino acids 59-80 of SEQ ID NO:1; and

Tyr-Gln-Thr-Lys-Val-Asn-Leu-Leu-Ser-Ala-Ile-Lys-Ser-Pro-Cys-Gln-Arg-Glu-Thr-Pro-Glu-Gly as amino acids 87-108 of SEQ ID NO:1.

24. A pharmaceutical composition, comprising an antibody according to claim 1, or fragment, region or pharmaceutically acceptable ester, ether, sulfate, carbonate, glucuronide or salt thereof, and a pharmaceutically acceptable carrier.

5           25. An isolated ~~TNF~~ polynucleotide, comprising a nucleotide sequence encoding an antibody according to claim 6, wherein said nucleotide sequence encodes at least one variable region in operable linkage with at least one constant region.

10           26. A polynucleotide according to claim 25, wherein said nucleotide sequence is selected from a genomic DNA sequence or a cDNA sequence.

          27. A polynucleotide according to claim 25, wherein said polynucleotide is an expression vehicle.

15           28. A host transformed or transfected with the polynucleotide according to claim 25.

          29. A host according to claim 28, wherein said host is a eukaryotic cell or a bacterial cell.

          30. A host according to claim 29, wherein said host is mammalian cell.

20           31. A process for preparing an antibody, fragment or region according to claim 6, comprising:

          (a) culturing a host according to claim 28 such that said antibody is expressed in recoverable amounts; and

25           (b) recovering said antibody, or a fragment or region thereof, from said host or culture.

          32. A method for treating an animal having a pathology mediated by a TNF comprising administering to said animal a therapeutic amount of a pharmaceutical composition according to claim 24.

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(33.) A method for treating an animal having a pathology mediated by a TNF comprising administering to said animal a therapeutic amount of a pharmaceutical composition according to claim 42.

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34. A method of removing from a sample a  $\text{TNF}\alpha$ , a fragment thereof, or an immune complex containing said  $\text{TNF}\alpha$ , the method comprising:

(a) contacting said sample to a device containing an antibody according to claim 1, or a fragment or region thereof, bound to a support, such that said  $\text{TNF}\alpha$ , portion thereof or immune complex reversible binds to said immobilized antibody, fragment or region to provide a bound  $\text{TNF}\alpha$ , portion or immune complex; and

(b) recovering said bound  $\text{TNF}\alpha$ , portion or immune complex from said bound antibody, fragment or region.

35. A method of removing from a sample a TNF, a fragment thereof, or an immune complex containing said TNF, the method comprising:

(a) contacting said sample to a device containing an antibody according to claim 6, or a fragment or region thereof, bound to a support, such that said TNF, portion thereof or immune complex reversible binds to said immobilized antibody, fragment or region to provide a bound TNF, portion or immune complex; and

(b) recovering said bound TNF, portion or immune complex from said bound antibody, fragment or region.

36. A method of treating an animal subject suspected of having a pathology or condition associated with elevated levels of TNF in a body fluid, comprising:

- (a) removing said TNF from said body fluid using a method according to claim 34; and
- (b) returning said body fluid to said animal.

37. A method of treating an animal suspected of having a pathology or condition associated with elevated levels of TNF in a body fluid, comprising:

- (a) removing said TNF from said body fluid using a method according to claim 35; and
- (b) returning said body fluid to said animal.

38. An immunoassay method for detecting human TNF in a sample, comprising:

- (a) contacting said sample with an antibody according to claim 1, or a fragment or region thereof; and
- (b) detecting the binding of the antibody to said TNF.

39. An immunoassay method for detecting human TNF in a sample, comprising:

- (a) contacting said sample with an antibody according to claim 6, or a fragment or region thereof; and
- (b) detecting the binding of the antibody to said TNF.

40. A method of treating an animal according to claim 32, wherein said pathology is selected from systemic lupus erythematosus, rheumatoid arthritis, sepsis syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, a bacterial infection, a viral infection, or a fungal infection.

41. A method of treating an animal according to claim 32, wherein said pathology is selected from alcohol-induced hepatitis, a chronic inflammatory pathology, a neurodegenerative disease, a vascular inflammatory pathology, a graft-versus-host pathology, Kaisaki's pathology and a malignant pathology.

42. A method of treating an animal according to claim 33, wherein said pathology is selected from sepsis syndrome, cachexia, circulatory collapse and shock resulting from acute or chronic bacterial infection, a bacterial infection, a viral infection, or a fungal infection, systemic lupus erythematosus, rheumatoid arthritis.

43. A method of treating an animal according to claim 33, wherein said pathology is selected from alcohol-induced hepatitis, a chronic inflammatory pathology, a neurodegenerative disease, a vascular inflammatory pathology, a graft-versus-host pathology, Kaisaki's pathology and a malignant pathology.

44. A method according to claim 41, wherein said neurodegenerative disease is selected from AIDS dementia complex, a demyelinating disease, multiple sclerosis, acute transverse myelitis, an extrapyramidal disorder, a cerebellar disorder, a lesion of the corticospinal system, a disorder of the basal ganglia, (a cerebellar disorder), a hyperkinetic movement disorder, Huntington's Chorea, senile chorea, a drug-induced movement disorder, a hypokinetic movement disorder, Parkinson's disease, progressive supranucleo palsy, a structural lesion of the cerebellum, a spinocerebellar degeneration, spinal ataxia, Friedreich's ataxia, a cerebellar cortical degeneration, a multiple systems degeneration, a systemic disorder, Refsum's disease, abetalipoproteinemia, ataxia telangiectasia, a mitochondrial multi-system disorder, a demyelinating core disorder, acute transverse myelitis, a disorder of the motor unit, a neurogenic muscular atrophy, anterior horn cell degeneration, amyotrophic lateral sclerosis, infantile spinal muscular atrophy, juvenile spinal muscular atrophy, Alzheimer's disease, Down's Syndrome, a diffuse Lewy body disease, senile dementia of Lewy body type, Wernicke-Korsakoff syndrome, chronic alcoholism, Creutzfeldt-Jakob disease,

subacute sclerosing panencephalitis, Hallerrorden-Spatz disease or dementia pugilistica.

45. A method according to claim 43, wherein said neurodegenerative disease is selected from AIDS dementia complex, a demyelinating disease, multiple sclerosis, acute transverse myelitis, an extrapyramidal disorder, a cerebellar disorder, a lesion of the corticospinal system, a disorder of the basal ganglia, a cerebellar disorder, a hyperkinetic movement disorder, Huntington's Chorea, senile chorea, a drug-induced movement disorder, a hypokinetic movement disorder, Parkinson's disease, progressive supranucleo palsy, a structural lesion of the cerebellum, a spinocerebellar degeneration, spinal ataxia, Friedreich's ataxia, a cerebellar cortical degeneration, a multiple systems degeneration, a systemic disorder, Refsum's disease, abetalipoproteinemia, ataxia telangiectasia, a mitochondrial multi-system disorder, a demyelinating core disorder, acute transverse myelitis, a disorder of the motor unit, a neurogenic muscular atrophy, anterior horn cell degeneration, amyotrophic lateral sclerosis, infantile spinal muscular atrophy, juvenile spinal muscular atrophy, Alzheimer's disease, Down's Syndrome, a diffuse Lewy body disease, senile dementia of Lewy body type, Wernicke-Korsakoff syndrome, chronic alcoholism, Creutzfeldt-Jakob disease, subacute sclerosing panencephalitis, Hallerrorden-Spatz disease or dementia pugilistica.

46. A method according to claim 44, wherein said neurodegenerative disease is multiple sclerosis.

47. A method according to claim 45, wherein said neurodegenerative disease is multiple sclerosis.

48. A pharmaceutical composition, comprising an antibody according to claim 6, or fragment, region or pharmaceutically acceptable ester, ether, sulfate, carbonate, glucuronide or salt thereof, and a pharmaceutically acceptable carrier.



49. A polynucleotide according to claim 25, wherein said nucleotide sequence comprises a polynucleotide which encodes said at least one variable region or said at least one constant region, wherein at least a portion of said polynucleotide hybridizes to at least a 15 base oligonucleotide complementary to the sequence presented in Figure 17A (SEQ ID NO:2) or Figure 17B (SEQ ID NO:3).

50. A method according to claim 32, wherein said animal is a human.

51. A method according to claim 33, wherein said animal is a human.

52. A method according to claim 34, wherein said animal is a human.

53. A method according to claim 37, wherein said animal is a human.

54. A method according to claim 40, wherein said pathology is rheumatoid arthritis.

55. A method according to claim 41, wherein said pathology is rheumatoid arthritis.

56. A method according to claim 32, wherein said pharmaceutical composition is administered in an amount of 0.1 to 50 mg/kg.

57. A method according to claim 33, wherein said pharmaceutical composition is administered in an amount of 0.1 to 50 mg/kg.